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Probing Radiation Damage in Protein Crystals with Micron-sized X-ray Beam

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X-ray-induced radiation damage to macromolecular crystals remains a major hindrance in diffraction data collection and interpretation of results in structural biology. Energy deposition by photoelectrons, emitted during irradiation of crystals, is thought to be a primary cause of radiation damage at data collection temperatures (~100 K). Photoelectrons are emitted preferentially along the polarization vector and according to Monte-Carlo simulations at 15 keV will be reabsorbed 4–4.5 microns from the point of emission. Subsequently, with a small enough beam, photoelectrons will carry energy outside the footprint of the beam resulting in less damage to the sample. Studies of radiation damage as function of beam size indicated that smaller beams indeed reduce damage to the diffracting volume of a protein crystal. Radiation damage as function of dose and of distance from the beam center was also mapped with a 1-μm beam at two energies: 15.1keV and 18.5 keV. Concurrent with the theories, the damage was higher along the polarization vector than in perpendicular direction, the width of the damaged area was 2–3 times larger than the incident beam size, and the damage did not transfer beyond 4–5 μm from the incident beam center.